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PROPERTY OFFICE

Ottawa Hull K1A 0G9

(21) (A1)	2,177,837
(86)	1995/12/05
(43)	1996/06/07

(51) Int.Cl. <sup>6</sup> C07K 14/715; A61K 38/20

(19) (CA) APPLICATION FOR CANADIAN PATENT (12)

(54) Interleukin-6 (IL-6) Antagonists Consisting in Soluble  
Forms of IL-6 Receptor  $\alpha$  Mutated in the GP130 Binding  
Interface

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(30) (IT) RM94A000794 1994/12/06

(57) 8 Claims

Notice: This application is as filed and may therefore contain an  
incomplete specification.



Industrie Canada Industry Canada

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Canada

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INTERLEUKIN-6 (IL-6) ANTAGONISTS CONSISTING IN SOLUBLE  
FORMS OF IL-6 RECEPTOR  $\alpha$  MUTATED IN THE GP130 BINDING  
INTERFACE

ABSTRACT

5           These       are       antagonists       of       interleukin-6,  
characterised in that they consist of soluble forms of  
the receptor  $\alpha$  of human IL-6 (shIL-6R $\alpha$ ) containing one or  
more mutation in the gp 130 binding interface. In a  
10       preferred embodiment, the mutations are present in a  
position chosen from the group comprising Ala 228, Asn  
230, His 280 and Asp 281. These antagonists can be used  
as agents capable of preventing and treating diseases  
caused by abnormal IL-6 activity.

15       Figure 4 shows the antagonist activity of the mutant  
Ala228Asp/Asn 230Asp/His280Ser/Asp281Val in comparison  
with the agonist properties of wild type shIL-6R $\alpha$ . This  
antagonist can be used for preparation of drugs for the  
prevention, control and treatment of diseases caused by  
abnormal IL-6 bioactivity.

20       (Fig. 4)

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## CLAIMS

1. Antagonist of interleukin-6 (IL-6), characterized in that it consists in soluble forms of the  $\alpha$  receptor of IL-6 (sIL-6R $\alpha$ ) containing one or more mutations in the interface binding with gp 130.  
5
2. Antagonist of interleukin-6 according to claim 1, in which sIL-6R $\alpha$  contains at least one mutation in a position chosen from the group comprising Ala 228, Asn 230, His 280 and Asp 281.
- 10 3. Antagonist of interleukin 6 according to claim 1 or 2, in which sIL-6R $\alpha$  contains the mutation Asn230Asp (SEQ ID NO: 1).
4. Antagonist of interleukin 6 according to claim 1 or 2, in which sIL-6R $\alpha$  contains the mutation  
15 Ala228Asp/Asn230Asp (SEQ ID NO: 2).
5. Antagonist of interleukin 6 according to claim 1 or 2, in which sIL-6R $\alpha$  contains the mutations His280Ser/Asp281Val (SEQ ID NO: 3).
- 20 6. Antagonist of interleukin 6 according to claim 1 or 2, in which sIL-6R $\alpha$  contains the mutations Ala228Asp/Asn230Asp/His280Ser/Asp281Val (SEQ ID NO: 4).
7. Use of interleukin 6 antagonists according to claims 1 to 6, for the study and preparation of drugs capable of controlling, preventing and treating diseases  
25 caused by abnormal IL-6 activity.
8. Soluble receptors acting as IL-6 antagonists and their use, as described, illustrated and claimed above.

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INTERLEUKIN-6 (IL-6) ANTAGONISTS CONSISTING IN SOLUBLE  
FORMS OF IL-6 RECEPTOR  $\alpha$  MUTATED IN THE GP130 BINDING  
INTERFACE

DESCRIPTION

5       The present invention relates to the field of  
immunology. More specifically, the subject of the  
present invention are mutants of the soluble forms of  
receptor  $\alpha$  of interleukin 6 that interfere in a negative  
10       manner in the formation of the dimeric receptor complex  
between the monomeric receptor complex IL-6/sIL-6R $\alpha$  and  
gp 130. A further subject of the invention is the use of  
these mutants as interleukin-6 antagonists to control,  
prevent and treat the diseases caused by abnormal IL-6  
activity.

15       The term "interleukin 6" or "IL-6", in the context  
of the present invention, means IL-6 and fragments,  
deletions, insertions, substitutions, mutations and  
modifications thereof that maintain the biological  
characteristics of wild IL-6. Unless otherwise  
20       specified, the term refers to human IL-6.

Interleukin-6 elicits a variety of biological  
responses on different target cells. However - while the  
physiological production of IL-6 regulates B-cells  
proliferation and maturation, T-cell activation and the  
25       production of acute-phase proteins in liver during  
inflammatory response - dysregulated production of the  
cytokine plays a crucial role in the pathogenesis of many  
inflammatory, autoimmune and neoplastic diseases.

As is known from the state of the art, various  
30       attempts have been made to perfect IL-6 bioactivity  
inhibitors, both in order to study the role played by  
this cytokine in the genesis of single diseases, and in  
order to define drugs for treatment.

It is also known that the mature human IL-6 protein  
35       (h IL-6) is a 185 amino acid polypeptide containing two  
disulfide bonds (Cys 45 - Cys 51, and Cys 74 - Cys 84).  
IL-6 functions through interaction of two binding sites

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(known as site I and site II) with at least two specific receptors (IL-6R $\alpha$  and gp 130) on the surface of the target cells, creating a trimeric complex IL-6-(receptor)<sub>2</sub>. This complex is formed sequentially: a first receptor (IL-6R $\alpha$ ) binds with low affinity to site I of IL-6 without transmitting the signal; subsequently a second receptor (gp 130), after binding with high affinity to site II of IL-6, transduces the signal.

Based on this mechanism, hIL-6 mutants have been designed that are capable of binding a first receptor to site I, but incapable of dimerising the receptor because of mutations that sterically inhibit binding of the second receptor to site II. Mutants of this type have been described in WO 94/09138 (Cetus Oncology Corporation), and WO 94/011402 and PCT/IT 94/00095 (Istituto di Ricerche di Biologia Molecolare P. Angeletti S.p.A.).

It has now surprisingly been found, and this discovery forms the basis of the present invention, that soluble forms of the receptor  $\alpha$  of IL-6 (sIL-6R $\alpha$ ) containing one or more mutations in the region interfacing with gp 130 are antagonists of interleukin 6.

In one embodiment, sIL-6R $\alpha$  contains at least one mutation in a position chosen from Ala 228, Asn 230, His 280 and Asp 281. The mutations may, for example, be chosen from the group comprising: Asn230Asp (SEQ ID NO:1); Ala228Asp/Asn230Asp (SEQ ID NO:2); His280Ser/Asp281Val (SEQ ID NO:3).

In a preferred embodiment, sIL-6R $\alpha$  contains multiple mutations in positions Ala 228, Asn 230, His 280 and Asp 281. A multiple mutation that has given good results is Ala228Asp/Asn230Asp/His280Ser/Asp281Val (SEQ ID NO:4).

The antagonists of IL-6, which according to the invention consist of soluble forms of the receptor  $\alpha$  of IL-6, mutated in the interface that binds to gp 130, can be administered at concentrations therapeutically effective for the treatment and prevention of diseases

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related to abnormal IL-6 activity. For this purpose, the antagonists according to the invention are administered preferably by endovenous and/or subcutaneous injection. Experts in the field are well acquainted with these methods of administration.

Up to this point a general description of the invention has been given. A more detailed description of the invention will now be provided, with the aid of the following examples, in order to give a clearer understanding of the objects, characteristics, advantages and operating methods thereof.

Figure 1A shows resolution on SDS/PAGE gel (12%), in vitro, of the co-immunoprecipitates of mutants according to the invention with sgp 130 (soluble form of gp 130) marked using  $^{35}\text{S}$ . Figure 1B shows the position on the gel corresponding to the migrated  $^{125}\text{I}$ -IL-6.

Figure 2 shows, in the form of a histogram, the different capacities of the wild type sIL-6R $\alpha$  receptor and the sIL-6R $\alpha$  receptors mutated to interact with gp 130 on the membrane of A 375 cells.

Figure 3 shows resolution on SDS/PAGE gel (12%), in vitro, of the co-immunoprecipitates of sgp 130 marked with  $^{35}\text{S}$  or with 2.0  $\mu\text{g}$  of wild receptor or with 2.0  $\mu\text{g}$  of mutant receptor (SEQ ID NO: 4).

Figure 4 shows the antagonist activity of the mutant SEQ ID NO: 4 on HepG2 cells in comparison with the agonistic properties of wild type shIL-6R $\alpha$ .

Figure 5 shows resolution on gel of the complex formed by APRF with the DNA binding site (SIE, Serum Inducible Element). (The mutant SEQ ID NO: 4 does not inhibit activation of OM-dependent APRF on HepG2 cells).

Antagonists of IL-6 according to the invention may be produced synthetically or using recombinant techniques. In the latter case the cDNA coding for sIL-6R $\alpha$  can be incorporated in a plasmid before being expressed in procaryotic or eukaryotic cells. Bacteria are the preferred procaryotic microorganisms.

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Alternatively, the cDNA coding for the sIL-6 $\alpha$  mutants according to the invention can be introduced into mammal cells: these mammal cells can be chosen from the group comprising CHO, COS, C127, HepG2, SK Hep. Furthermore, the protein can also be expressed in insect cells (Sf9 or HighFive) using the well known recombinant Baculovirus technology (AcNPV strain).

#### Example 1

##### Preparation of sIL-6 $\alpha$ mutants

sIL-6 $\alpha$  cDNA was obtained as a 5'EcoRI-3'XbaI fragment by PCR using the IL-6 $\alpha$  complete cDNA as template (Sporeno E., Paonessa G., Salvati A.L., Graziani R., Delmastro P., Ciliberto G. and Toniatti C. (1994) J. Biol. Chem. 269, 10991-10995). The 3' primer was designed in order to introduce an artificial TAG stop codon at amino acid position 324 preceded by a six-histidine coding sequence. This was done to ensure that the sIL-6 $\alpha$  and the mutants thereof produced by us have a six histidine "tail" at the carboxy-terminal end of the molecule, which can be useful for purification of the molecule using metal affinity chromatography. The fragment generated was then introduced into the COS-7 cells expression vector pCDNAI (Invitrogen) and sequenced in its entirety, thus obtaining plasmid pC6FRH.

Plasmid pC6FRH was in turn used as a template to obtain constructs coding for the following four mutants: Asn230Asp (SEQ ID NO: 1); Asn230Asp/Ala228Asp (SEQ ID NO: 2); His280Ser/Asp281Val (SEQ ID NO 3) and Ala228Asp/Asn230Asp/His280Ser/Asp281Val (SEQ ID NO: 4), by a two-step PCR as described in the past (Landt O., Grunert H.P., and Hahn, U. (1990) Gene 96, 125-128).

The mutants were then expressed in COS-7 cells. For this purpose, the COS-7 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FCS, plus glutamine and antibiotics, at 5% CO<sub>2</sub>. For protein expression, 2.5x10<sup>6</sup> COS-7 cells were seeded in 100-mm tissue culture dishes and the next day transfected

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with 2  $\mu$ g of the various shIL-6R $\alpha$  expression vectors using the DEAE-dextran technique as described in Seed B., Aruffo A. (1987) Proc. Natl. Acad. Sci. USA 84, 3365-3369. 16 h after transfection cells were split, replated  
5 in 100-mm dishes and grown in complete medium at 37°C. After 72-96 h the medium was collected, centrifuged and used for co-immunoprecipitation experiments and binding analysis. To monitor the expression level of each  
10 mutant,  $2.5 \times 10^5$  transfected COS-7 cells were replated in 35-mm dishes and, 48 h after transfection, metabolically labeled with [ $^{35}$ S]methionine for 4h. The supernatants were immunoprecipitated with anti-human IL-6R $\alpha$  monoclonal antibody I6R1/9.G11. The immunoprecipitated material was then analyzed by electrophoresis through a polyacrylamide  
15 gel containing SDS.

#### IL-6 binding analysis of mutants

To determine the affinity for IL-6 of the sIL-6R $\alpha$  mutants contained in the conditioned COS cells culture medium, the six-histidine "tail" in the proteins produced  
20 by us was used.

Appropriate amounts of transfected COS cells supernatant, previously determined in titration experiments and to which imidazole was added up to a final concentration of 5 mM, were mixed with 20-40 pM  
25  $^{125}$ I-IL-6 and increasing concentrations of unlabeled cytokine. At equilibrium conditions, 40  $\mu$ l of Ni $^{2+}$ -NTA-Agarose (Quiagen), a resin capable of selectively binding proteins containing histidine "tails", was added and incubation prolonged for one additional hour. The  
30 ligand bound by the receptor and, therefore, indirectly resin-associated, was separated from free ligand (supernatant) by centrifugation through a cushion of 30% sucrose in PBS. All steps were performed at 4°C. The values of free and bound cpm (radioactivity count per  
35 minute) allowed drawing of competition displacement curves. The apparent Kd of the various soluble IL-6 receptors was determined after Scatchard transformation



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of the results (see the article by Sporeno, Paonessa, Salviati, Graziani, Delmastro, Ciliberto and Toniatti (1994), J. Biol. Chem. 269, 10991-10995). Analysis of binding data and curve fitting was done using the  
5 UltraFit software (Biosoft®) on a Macintosh computer.

As can be seen from Table 1, the binding affinity:  
1) remains substantially unprejudiced for mutants Asn230Asp (SEQ ID NO: 1), Asn230Asp/Ala228Asp (SEQ ID NO: 2); b) is reduced in a slight but reproducible manner  
10 (between 2.5 nM and 6 nM) for His280Ser/Asp281Val (SEQ ID NO: 3); and c) the activity of the wild type form is fully maintained for the mutant Ala228Asp/Asn230Asp/His280Ser/Asp281Val (SEQ ID NO: 4).

15

TABLE 1

IL-6 binding affinity of soluble interleukin 6 receptor (wild and mutated), labeled with histidine and expressed in COS cells

Mutant	IL-6 binding affinity
	nM
Asn230Asp	(SEQ ID NO: 1) 1.5 ± 1
Ala228Asp/Asn230Asp	(SEQ ID NO: 2) 2.0 ± 1
His280Ser/Asp281Val	(SEQ ID NO: 3) 4.3 ± 2
25 Ala228Asp/Asn230Asp/	
His280Ser/Asp281Val	(SEQ ID NO: 4) 2.5 ± 1
wild type sIL-6Rα	2.0 ± 1

Binding of mutants to gp130

The IL-6-dependent formation of sIL-6Rα/sgp130  
30 heterodimers can be easily monitored in vitro by co-immunoprecipitations with the use of suitable monoclonal antibodies. A selection was therefore made of the mutants showing an IL-6 binding affinity in the same order of magnitude as the wild-type and their binding to  
35 sgp130 was evaluated by co-immunoprecipitation in the presence of IL-6.

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<sup>35</sup>S-labelled sgp 130 was incubated with <sup>125</sup>I-IL-6 (as an internal standard for the immunoprecipitation) and aliquots of transfected COS-7 cell culture medium, containing either native or mutant receptors. After in vitro binding, anti IL-6Rα mAb I6/R19.G11 was added to the mixtures and the immunoprecipitates resolved on SDS/PAGE gels. The results are shown in figure 1. As can be seen, the mutant His280Ser/Asp281Val (SEQ ID NO: 3) strongly reduced the association of the IL-6/ sIL-6Rα complex sgp 130 molecule (lane 4). Also the single substitution mutant Asn230Asp (SEQ ID NO: 1) had decreased interaction with sgp130 (lane 2). The effect of this mutant on interaction with sgp 130 is of the same magnitude as that of mutant Ala228Asp/Asn230Asp (SEQ ID NO: 2). Finally, the quadruple mutant Ala228Asp/Asn230Asp/His280Ser/Asp281Val (SEQ ID NO: 4) displayed the lowest ability to co-immunoprecipitate <sup>35</sup>S-gp 130 (lane 5).

The receptor mutants were also tested for their ability to interact with gp130 on the cell surface. For this purpose, binding experiments were performed on human melanoma A375 cells which have an excess of gp130 molecules over IL-6Rα. In fact, binding of <sup>125</sup>I-IL-6 to A375 monolayers could be strongly enhanced by addition of the soluble receptor. This phenomenon is due to the ability of the sIL-6Rα to bind <sup>125</sup>I-IL-6 and subsequently to interact with the gp130 molecules present on the surface of the cells. The specificity of this interaction is demonstrated by the fact that the increased binding of <sup>125</sup>I-IL-6 in the presence of sIL-6Rα is competed by the addition of an excess of unlabeled human Oncostatin M (OM) (fig. 2). This cytokine is able to bind directly to gp130 and to compete the binding of the IL-6/IL-6Rα complex with gp130. Unlike wild type shIL-6Rα, only a minor increase in specific binding was detected when cells were challenged with <sup>125</sup>I-IL-6 plus the soluble mutant receptors acting as antagonists

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(figure 2). The greatest disturbance to interaction with gp130 is shown by the mutants His280Ser/Asp281Val (SEQ ID NO: 3) and Ala228Asp/Asn230Asp/His280Ser/Asp281Val (SEQ ID NO: 4).

5 Demonstration that the mutant Ala228Asp/Asn230Asp/His280Ser/Asp281Val (SEQ ID NO: 4) is an IL-6 antagonist

The results shown above indicate this mutant to be the one that, without any decrease in the affinity for IL-6, has the greatest effect on gp130 binding. In order  
10 to study the biological activity of this mutant, its production and that of the wild type soluble receptor was scaled up using the MaxBac® system (Invitrogen's Baculovirus Expression System). The expressed and purified receptors were tested in immunoprecipitation  
15 experiments.

Sf9 cells, grown in Grace's insect medium, were used for transfection of transfer vectors, isolation of recombinant virus and preparation of high-titer virus stocks. High Five® cells (Invitrogen) were instead used  
20 for production of proteins. Briefly,  $4 \times 10^7$  High Five® cells, grown in complete Grace's insect medium, were seeded into 750 ml flasks and infected with the appropriate recombinant virus at a MOI of 10. After 2 hours, cells were washed and SF-900 serum-free medium was added. The culture supernatants were harvested at 36  
25 hours post-infection, dialyzed against PBS and directly loaded on a  $\text{Ni}^{2+}$ -NTA-agarose column. After washing steps with PBS/8 mM imidazole, both wild type shIL-6R $\alpha$  and mutant were eluted in PBS/80 mM imidazole. Purified  
30 protein was dialyzed against PBS and directly used or stored at 4°C for up to three weeks.

The use of purified proteins made it possible to quantify the amount of receptor and to compare the ability of wild type receptor and mutant receptor to  
35 interact with sgpl30 in the presence of a wide range of IL-6 concentrations. The results (figure 3) were in good agreement with those previously obtained with COS cell-

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5 derived receptors. Interestingly at each point of the curve the amount of co-immunoprecipitated gp130 is lower for the mutant receptor than for the wild type receptor and this is true even at the highest concentrations of the cytokine tested (100 nM). These findings confirm that the interaction between IL-6 and mutant sIL-6R $\alpha$  generates a complex with lower affinity for gp130.

10 To test the potential antagonism of the mutant for IL-6 activity, we chose the IL-6 dependent activation of transcription factor APRF (Acute Phase Response Factor)/STAT 3 in human hepatoma cells. Activation of APRF, which is dependent on tyrosine phosphorylation, is a rapid cytoplasmic event which takes place within minutes of stimulation with all cytokines of the IL-6 family.

15 Human HepG2 cells were stimulated with increasing concentrations of IL-6 together with a fixed amount of wild type and mutant receptors (100 nM). As shown in figure 4, while shIL-6R $\alpha$  potentiated APRF induction by IL-6 (compare lanes 2-4 and 5-7 in figure 4), the soluble mutant receptor not only lacked any agonistic activity but instead down-modulated the activity of the cytokine (figure 4, lanes 8-10). As predicted from the co-immunoprecipitation experiments, the inhibition was complete when cells were treated with up to 20 pM IL-6 (lanes 8 and 9, figure 4) and only a weak induction was detected at the highest concentration of IL-6 (200 pM, lane 10 of figure 4).

25 To test if antagonism was specific for IL-6, the mutant was added to HepG2 cells induced with the IL-6-related cytokine OM, which is also known to efficiently induce APRF phosphorylation. As shown in figure 5, the mutant did not antagonize OM activity.

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## SEQUENCE LISTING

## GENERAL INFORMATION

- (i) APPLICANT: ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE P. ANGELETTI S.p.A.
- 5 (ii) TITLE OF INVENTION: Interleukin-6 (IL-6) antagonists consisting in soluble forms of IL-6 receptor  $\alpha$  mutated in the gp 130 binding interface
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
- 10 (A) ADDRESSEE: Società Italiana Brevetti  
(B) STREET: Piazza di Pietra, 39  
(C) CITY: Rome  
(D) COUNTRY: Italy  
(E) POSTAL CODE: I-00186
- 15 (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk 3.5" 1.44 MBYTES  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS Rev. 6.22  
(D) SOFTWARE: Microsoft Word 6.0
- 20 (viii) ATTORNEY INFORMATION  
(A) NAME: DI CERBO, Mario (Dr.)  
(C) REFERENCE: RM/X88470/PC-DC
- (ix) TELECOMMUNICATION INFORMATION
- 25 (A) TELEPHONE: 06/6785941  
(B) TELEFAX: 06/6794692  
(C) TELEX: 612287 ROPAT
- (1) INFORMATION FOR SEQ ID NO: 1:
- (i) SEQUENCE CHARACTERISTICS
- 30 (A) LENGTH: 15 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: no
- (v) FRAGMENT TYPE: internal
- 35 (vii) IMMEDIATE SOURCE:  
(A) SYNTHESIS: production as recombinant protein in eucariotic cells

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- 11 -

(ix) FEATURE:  
 (A) NAME: Asn230Asp  
 (C) IDENTIFICATION METHOD:  
 Electrophoresis on a denaturing SDS/polyacrylamide gel  
 (D) OTHER INFORMATION: amino acid  
 sequence of a mutant form of the interleukin 6 receptor  
 from position 222 to position 236.  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:  
 Ile Thr Val Thr Ala Val Ala Arg Asp Pro Arg Trp Leu Ser Val  
 1 5 10 15  
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 (A) LENGTH: 15 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: protein  
 (iii) HYPOTHETICAL: no  
 (v) FRAGMENT TYPE: internal  
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 protein in eucariotic cells  
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 (C) IDENTIFICATION METHOD:  
 Electrophoresis on a denaturing SDS/polyacrylamide gel  
 (D) OTHER INFORMATION: amino acid  
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 from position 222 to position 236.  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:  
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 1 5 10 15  
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 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: protein

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(iii) HYPOTHETICAL: no  
(v) FRAGMENT TYPE: internal  
(vii) IMMEDIATE SOURCE:  
(A) SYNTHESIS: production as recombinant  
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(ix) FEATURE:  
(A) NAME: His280Ser/Asp281Val  
(C) IDENTIFICATION METHOD: Electrophoresis  
10 on a denaturing SDS/polyacrylamide gel  
(D) OTHER INFORMATION: amino acid  
sequence of a mutant form of the interleukin 6 receptor  
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:  
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(4) INFORMATION FOR SEQ ID NO: 4:  
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(B) TYPE: amino acid  
20 (D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: protein  
(iii) HYPOTHETICAL: no  
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(vii) IMMEDIATE SOURCE:  
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protein in eucariotic cells  
(ix) FEATURE:  
(A) NAME:  
Ala228Asp/Asn230Asp/His280Ser/Asp281Val  
30 (C) IDENTIFICATION METHOD: Electrophoresis  
on a denaturing SDS/polyacrylamide gel  
(D) OTHER INFORMATION: amino acid  
sequence of a mutant form of the interleukin 6 receptor  
from position 222 to position 287.  
35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:  
Ile Thr Val Thr Ala Val Asp Arg Asp Pro Arg Trp Leu Ser Val Thr  
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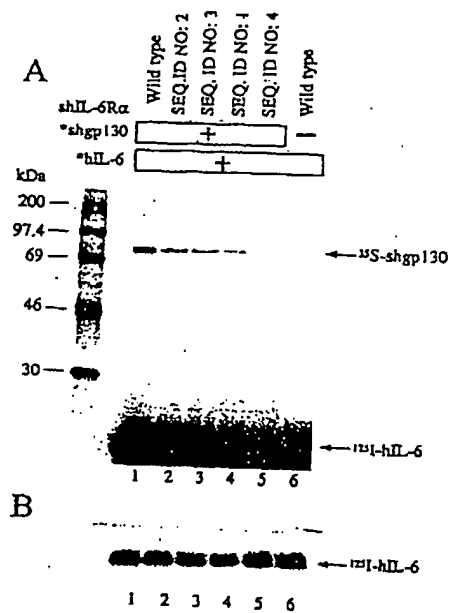
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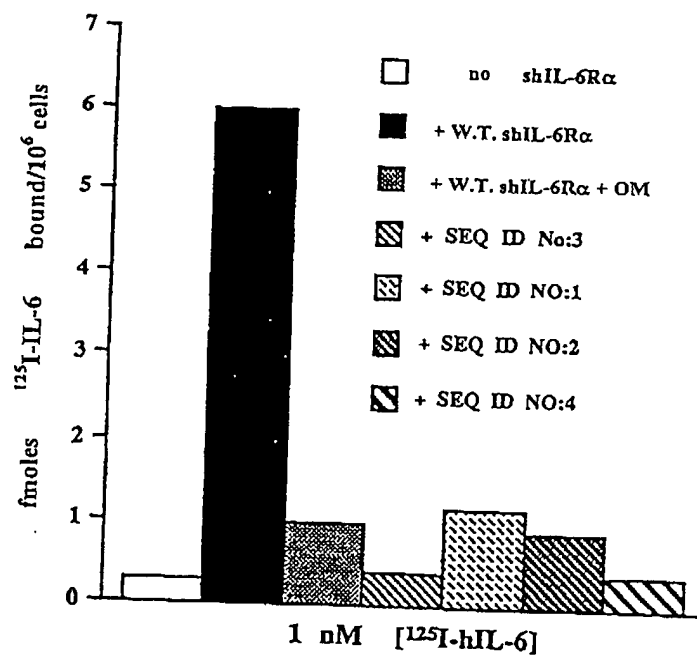
Fig. 1



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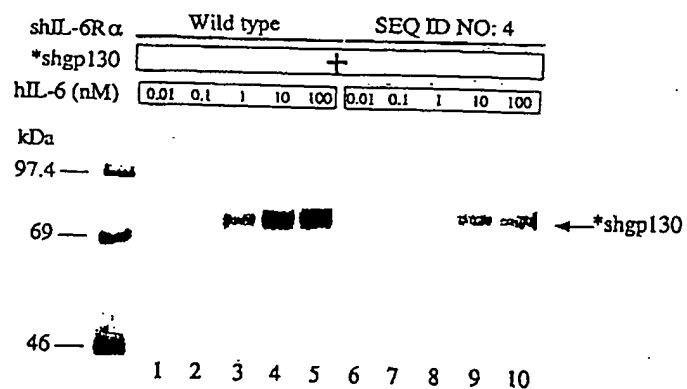
FIG. 2



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Fig. 3

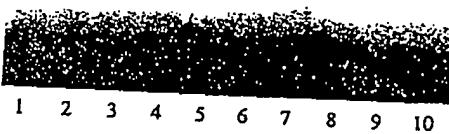



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Fig. 4

	Wild type						SEQ ID NO:4		
shIL-6R $\alpha$ (100 nM)	—	—	—	—	+	+	+	+	+
hIL-6 (pM)	—	2	20	200	2	20	200	2	20

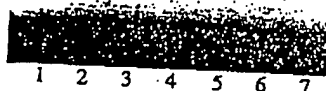



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Fig. 5

shIL-6R $\alpha$ (100 nM)	-	-	Wild type	SEQ ID NO: 4	-	Wild type	SEQ ID NO: 4
hIL-6 (200 pM)	-	+	+	+	-	-	-
hOM (200 pM)	-	-	-	-	+	+	+



1 2 3 4 5 6 7